

Standard operasjonsprosedyre: Weaning of pups in mice and rat breeding

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WEANING OF PUPS IN MICE AND RAT BREEDING

1. PURPOSE

- 1.1 Mouse and rat pups are separated from the parents before a new litter is born in order to avoid overcrowded cages and unnecessary strain on the mother and to ensure that younger pups have a good chance of survival.
- 1.2 Good animal welfare must be ensured for the newly separated pups.

2. DIVISION OF RESPONSIBILITY

- 2.1 KPM separate pups from breeding cages housed in KPM (MDU, the Barrier, KPMe and Conventional unit) from Monday to Friday. Litters are usually not separated at weekends and on public holidays but can be, if there is sufficient staff capacity.
- 2.2 The user must send a clear message to KPM if single males/females in a litter are to be kept. These must meet the criteria set out in point 3.3 below.

3. PROCEDURE

- 3.1 Mice pups are separated on day 20 after birth. Rats are separated on day 21 after birth. Breeding females can become pregnant immediately after giving birth. Mice can fall pregnant from between 18 to 21 days after giving birth, depending on the strain. Pregnancy in rats often lasts one or two days longer than in mice.
- 3.2 The oldest litter of one female must be separated before a new litter is born so that the female can look after the new-borns in the best possible way. If the pups are small or weak, they can stay with the mother longer than 20/21 days, if there is little chance that the female will give birth to a new litter. The pups are given wet feed. Update the separation date on the breeding cage.
- 3.3 If there is only one male or female in the litter, it will be euthanized by time of separation, unless the individual concerned is a newly established line or is very important for other reasons. The user must notify KPM if a litter is critical for the survival of the strain. Young animals should not be housed alone since this leads to a great risk of stress. Males can't be placed with other males due to fighting. Females from different breeding cages should not be placed together due to different pedigrees/birth dates. Young females placed with females that are somewhat older may also be poorly received. If single females are to be placed together with other females, these must be identifiable by means of different ear tagging.



- 3.4 The gender of the pups is determined at time of cage change and checked again at the time of separation. The sex can be differentiated by looking at the distance from the anus to the penis/vagina (urogenital distance). Males have a longer urogenital distance than females. The sex of pigmented mice, especially in younger and newborn mice, can also be differentiated by observing anogenital pigmentation (see illustration 1): males have a distinct, darker spot at the perineum than females.
- 3.5 Newly separated pups are recorded immediately in Science Linker (SL). Make sure that a tag request is created if requested. Other notes on the breeding cage card must be inspected and comments on phenotype and environmental enrichment must be copied onto the cage card for the pups. Males are given blue cage cards and females pink cage cards. Pups of the same sex from the same litter should not be split up if they can be housed in the same cage (see table below for housing mice and rats). Exceptions to this rule should be recorded under "Notes" on the breeding cage card.
- 3.6 If two litters are born very close together and you cannot see the difference between them, they should be separated as one litter. If there are two litters recorded in Science Linker, the males should be separated as one litter with the same date of birth and the females as the other litter.
- 3.7 The pups should be separated as follows: GM500 = max 5 mice, GM900 = 6-11 mice, GR900 = max 4 rats. The cages are placed inside the rack intended for the user group.
- 3.8 Sufficient enrichment, for example extra paper if needed, and a tunnel/igloo must be provided. Provide extra food but do not overfill the food basket. Make sure that the food basket is properly positioned, otherwise animals might get caught in the food basket. Some food should be placed on the bottom of the cage to help the pups feed during the first few days. Male mice should be given some paper from the breeding cage to minimize future fighting. Make sure a running wheel is provided if described on the cage card. One small water bottle is placed in GM500 cages and two big water bottles are placed in GM/GR900 cages.
- 3.9 All newly separated mice and rats are to be given wet food in a petri dish. The food must have been soaked in autoclaved water before placed in the dish. Top off with water from the bottle given to the pups. Do not overfill the dish. Change the wet food every day or every other day.
- 3.10 If the pups are clearly small, these must be closely followed up. The cage must be marked with cage number, date and "Give wet food" on a pink note. The wet food are to be replaced every day and a close inspection of the animals are given. Be especially careful that small rat pups get enough wet food. If needed, give two dishes of wet food. If any of the young are very small and weak during separation, they must be euthanized.
- 3.11 Teeth must be inspected. In case of malocclusion, the animal must be euthanized. Observe for any abnormalities, this should be registert in SL in "notes". Typical abnormalities is: Eyes, tail etc.
- 3.12 Make sure that the tunnel / igloo is placed under the water bottle in GM900-cages (mice). Newly separated pups may have difficulty reaching the water bottle in these cages. Mark the cage so that this is followed up until the young are big enough.

4. HEALTH, SAFETY AND ENVIRONMENT (HSE)

- 4.1 Everyone must have adequate training to ensure they use the proper clothing and protective gear.
- 4.2 Everyone who handles animals must have adequate training and practice to ensure the proper handling of animals.
- 4.3 The work must take place under a ventilated bench or LAF bench to avoid exposure to and spread of allergens and potential pathogens.
- 4.4 Everyone who handles chemicals must have adequate training and access to proper protective gear to ensure the safe use of these chemicals.
- 4.5 Everyone should be familiar with the Eco Online and Safety Data Sheets for the chemicals they may be exposed to.

5. EQUIPMENT AND MAINTENANCE

- 5.1 GM500, GMGR900
- 5.2 Enrichment: paper, tunnel, igloo, running wheel
- 5.3 Autoclaved food and water
- 5.4 Cage cards
- 5.5 Petri dish

6. HISTORY OF EDITING

- 6.1 24.10.2010: SOP written for the «old» department.
- 6.2 15.11.2012: SOP transferred to the new template. This must be revised when the database for the new department has been chosen.
- 6.3 03.03.2014 Revised SOP to amend the number of animals per cage etc.
- 6.4 08.10.2018 Substantial changes (Frøydis Lie Kilmer)
- 6.5 31.07.2020: Some smaller changes, e.g. anogenital pigmentation in pups. (Frøydis Kilmer)
- 6.6 09.02.2021: All weaned pups are to be given wet food. Tunnel/ igloo must be placed under the bottle in 900- cages (mice). (Frøydis Kilmer)
- 6.7 12.04.2023: Smaller changes. (Erle Linnea Fevåg)

7. REFERENCES

- 7.1 Forskrift om forsøk med dyr (Regulation on animal experiments) last amended FOR-2010-08-06-1147
- 7.2 APPENDIX A to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. Guidelines for the accommodation and care of animals
- 7.3 DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010 on the protection of animals used for scientific purposes
- 7.4 <https://www.nature.com/articles/labn0109-35.pdf?proof=true>

The housing of mice and rats

Mice

GM500 cages	GM900 cages
1-5 mice	Max. 11 mice

Rats

Weight	GM900 cages	GM1800 cages
< 200 g	2-4 rats	Max. 7 rats
200-300 g	2-3 rats	Max. 7 rats
300-400 g	2 rats	Max. 5 rats
400-600 g	2 rats	Max. 4 rats
> 600 g	Not allowed	Max. 3 rats

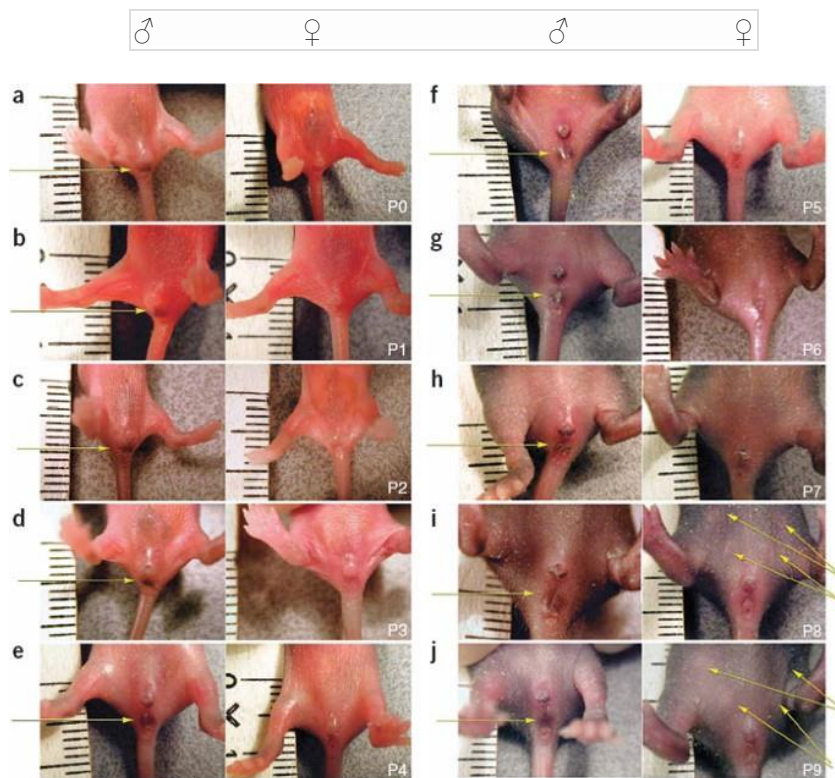


Illustration 1: Sex differentiation in pups with anogenital pigmentation.